SUPPLEMENTAL INFORMATION

Zika, chikungunya, and dengue virus detection. The primers and probes included in the internally-controlled Zika, chikungunya and dengue (IC-ZCD) assay have been described previously.[1] The assay for RNase P detection as a heterologous, intrinsic internal control has also been described.[2] RNase P primers and probes were used at concentrations published during development of the pan-dengue virus (DENV) assay, with which the IC-ZCD assay was designed to be compatible.[2] The 5′ fluor and 3′ quencher for the probes in the IC-ZCD assay (Biosearch Technologies, Novato, CA) were as follows: DENV, fluorescein, Black Hole Quencher (BHQ)-1; RNase P, Cal Fluor 540, BHQ-1; ZIKV, Cal Fluor 610, BHQ-2; CHIKV, Quasar 670, BHQ-2.

RNA was extracted from all samples and specimen types with the QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA) using 140 μL of sample and a 60-μL elution volume. All IC-ZCD reactions were performed on a Cobas Z 480 instrument (Roche, Basel, Switzerland) using 25-μL reactions of the SuperScript III Platinum One-Step qRT-PCR kit (Life Technologies, Carlsbad, CA) and 5 μL of RNA template. Cycling conditions for the IC-ZCD assay were the following: 52°C for 15 minutes; 94°C for 2 minutes; 45 cycles of 94°C for 15 seconds, 55°C for 20 seconds (acquisition), and 68°C for 20 seconds. Each run included a no-template control, a negative control (positive for RNase P but negative for any pathogen), and positive controls for ZIKV, CHIKV, and DENV. To account for cross-talk between channels on the Cobas Z 480 instrument (Roche Molecular Systems, Pleasonton, CA), a color compensation file was created for the IC-ZCD assay. The color compensation file was created by testing monoplex reactions for each target in triplicate. For ZIKV, CHIKV, and DENV, quantified single-stranded DNA containing the target sequence was tested at 6.0 $\log_{10}$ copies/μL of eluate.[1] For RNase P, an archived clinical sample was run in triplicate. This sample was obtained before the ZIKV outbreak in Ecuador, and tested negative for DENV and CHIKV using commercial molecular assays (DENV Simplexa and Detection Kit CHIK Virus; Tib Molbiol, Berlin, Germany, respectively).

Additional cerebrospinal fluid (CSF) testing, Case 3. Testing of CSF for additional pathogens included the following: gram stain, negative; real-time polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*, not detected; real-time PCR for herpes viruses, not detected (herpes simplex virus 1, herpes simplex virus 2, Epstein–Barr virus, varicella zoster virus, cytomegalovirus, human herpes virus 6).

SUPPLEMENTAL REFERENCES

1. Waggoner JJ, Gresh L, Mohamed-Hadley A, Ballesteros G, Davila MJ, Tellez Y, Sahoo MK, Balmaseda A, Harris E, Pinsky BA, 2016. Single-reaction multiplex reverse transcription PCR for detection of Zika, chikungunya, and dengue viruses. *Emerg Infect Dis 22:* 1295–1297.
2. Waggoner JJ, Abeynayake J, Sahoo MK, Gresh L, Tellez Y, Gonzalez K, Ballesteros G, Balmaseda A, Karunaratne K, Harris E, Pinsky BA, 2013. Development of an internally controlled real-time reverse transcriptase PCR assay for pan-dengue virus detection and comparison of four molecular dengue virus detection assays. *J Clin Microbiol 51:* 2172–2181.